Materials and methods

As a model process for cell-free protein synthesis a commercial available kit was used to produce a fluorescence protein which can easily be detected by a fluorescent reader. The concentrations of other relevant process reagents were measured over time to estimate the reaction kinetic parameters of the protein for a computational modeling approach.

Results and discussion

Our studies revealed an ATP consumption of 1.67E13 ATP molecules/second to maintain a production rate of 14,05E11 molecules/second for an active fluorescence protein. From this data, the required constraints of a regeneration area were calculated. However, the effects of spatial distribution and mass transport limitations are neglected. By further integrating these factors into the computational model, an optimized geometry of a regeneration module can be derived by our simulations.

doi:10.1016/j.bbabio.2012.06.051

1P19

ATP-driven H^+ pump activity of thermophilic *Bacillus* PS3 F_oF_1-ATP synthase reconstituted in a liposomal membrane measured by fluorescence ensemble and single liposome assays

Y. Kasuya, N. Soga, T. Suzuki, M. Yoshida, K. Kinosita Jr.
Waseda University, Faculty of Science and Engineering, Department of Physics, 3-4-1 Ohkubo, Shinjuku-ku, Tokyo 169-8555, Japan; Kyoto Sangyo University, Faculty of Life Sciences, Department of Molecular Bioscience, Kamigamo-Motoyama, Kyoto 603-8555, Japan
E-mail: y.kasuya@aoni.waseda.jp

F_oF_1 ATP synthase is composed of a water soluble F_1 portion that has three catalytic sites for ATP synthesis/hydrolysis reaction and a membrane embedded F_o portion that acts as a H^+ channel. The F_oF_1 synthesizes ATP using the energy of H^+ flow through the F_o portion, and pumps H^+ in the reverse direction when ATP is hydrolyzed in the F1 portion. ATP hydrolysis and resultant subunit rotation in F_1 have been well characterized by various single molecule techniques. The transport of H^+ through F_o, in contrast, has been difficult to observe in single molecules, and bulk assays have in most cases qualitative rather than quantitative.

Here, we measured the H^+ pump activity of F_oF_1 from thermophilic *Bacillus* PS3 (TF_oF_1) by an ensemble assay in a spectrophotometer and a single liposome assay under a microscope. To visualize the H^+ transport through the TF_oF_1 complex into liposomes driven by ATP hydrolysis, a fluorescent dye pH-rodo, of which the fluorescence intensity increases upon lowering of pH, was conjugated to the headgroup of phospholipids. The dye-labeled liposomes containing TF_oF_1 responded to the addition of ATP by gradually increasing the fluorescence. Both the rate of the initial rise and the final plateau level became higher at higher ATP concentrations. No fluorescence increase was observed for liposomes without the protein or in the TF_oF_1-liposome pretreated with an inhibitor *N*,*N*'-dicyclohexylcarbodiimide. By calibrating the buffering capacity, we estimated the number of pumped protons per TF_oF_1. The maximal initial rate, V_{max}, was in the order of 600 H^+ sec^{-1} in ensemble assays and 800 H^+ sec^{-1} in single-liposome assays where each liposome contained ~one TF_oF_1 molecule on average, for the starting pH of 8.0 and at room temperature. The Michaelis constant, K_m, for ATP was ~20 μM in both assays. The ATPase activity measured by NADH oxidation coupled to ATP regeneration showed V_{max} of ~200 ATP hydrolyzed per second, consistent with the H^+/ATP ratio of 10/3 expected for TF_oF_1. Now we are still trying to determine the H^+ pump activity more precise.

doi:10.1016/j.bbabio.2012.06.052

1P20

Reconstitution of Vacuolar type rotary H^+-ATPase/synthase from *Thermus thermophilus*.

J. Kishikawa, K. Yokoyama
Department of Molecular Biosciences, Faculty of Life Sciences, Kyoto Sangyo University, Motoyama, Kamigamo, Kyoto, Japan
E-mail: kishi.jun@cc.kyoto-su.ac.jp

Vacuolar type rotary H^+-ATPase/synthase (V_oV_1) from *Thermus thermophilus*, composed of nine subunits, A, B, D, F, C, E, G, I, and L, has been reconstituted from individually isolated V_1 ($A_3B_3D_1F_1$) and V_o ($C_1E_2G_2I_1L_{12}$) subcomplexes *in vitro*. A_3B_3D and A_3B_3 also reconstituted with V_o, resulting in a holoenzyme-like complexes. However, A_3B_3D-V_o and A_3B_3-V_o did not show ATP synthesis and dicyclohexylcarbodiimide (DCCD) sensitive ATPase activity. The reconstitution process was monitored in real time by fluorescence resonance energy transfer (FRET) between an acceptor dye attached to subunit F or D in V_1 or A_3B_3D, and a donor dye attached to subunit C in V_o. The estimated dissociation constants K_d for V_oV_1 and A_3B_3D-V_o were ~0.3 nM and ~1 nM at 25°C, respectively. These results suggest that the A_3B_3 domain tightly associated with the two EG peripheral stalks of V_o, even in the absence of the central shaft subunits. In addition, F subunit is essential for coupling of ATP hydrolysis and proton translocation and has a key role in the stability of whole complex. However the contribution of the F subunit to the association of A_3B_3 with V_o is much lower than that of the EG peripheral stalks.

doi:10.1016/j.bbabio.2012.06.053

1P21

A hydrophilic environment enhances opening and deprotonation of the binding sites in the membrane rotor of a mitochondrial ATP synthase

Alexander Krah, José D. Faraldo-Gómez
Max Planck Institute of Biophysics, Theoretical Molecular Biophysics Group, Max-von-Laue-Strasse 3, 60438 Frankfurt am Main, Germany
E-mail: alexander.krah@biophys.mpg.de

A recent crystal structure of the c-subunit ring from the *S. cerevisae* ATP synthase reveals its ten proton-binding sites in a seemingly open conformation. This is in contrast to known structures of bacterial c-rings, in which the ion-binding sites are consistently seen in the closed form. This observation raises the possibility that the mechanisms of mitochondrial c-rings are somehow distinct from their bacterial homologues, perhaps due to a different architecture or amino-acid sequence.

Here, we use molecular simulations to demonstrate that the open-state observed in the yeast c10 rotor is induced by the crystallization buffer. This buffer, primarily consisting of 2-methyl-2,4-pentanediol (MPD) in water, provides an environment for the protein that is very different from the detergent-based buffers used previously. Like detergent, MPD coats the transmembrane hydrophobic surfaces of the protein. However, MPD molecules can also form H-bonds with polar moieties of the protein. In addition, MPD is water permeable, and thus exposed hydrophilic regions on the c-ring surface, including the ion-binding sites, are well hydrated in the crystal.

Our simulations show that the possibility of interactions with water and MPD energetically favors the opening of the proton-binding sites. By contrast, when we simulate the ring embedded in a hydrophobic environment, its binding sites preferably adopt a closed state, in analogy with its prokaryotic counterparts. Therefore, the